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(54) Process for extracting polyphenolic antioxidants from purine-containing plants

(57) A process for isolating polyphenolic antioxidants from purine-containing plant material, comprising the steps of:

(a) extracting the plant material with an hydroxylic extracting liquid preferably at low temperature;
(b) adsorbing the extract obtained in step (a), preferably at low temperature;
(c) desorbing the adsorbed material with a hydroxylic desorbing liquid, preferably at relatively high temperature; and
(d) concentrating and optionally drying the desorbed material.

The process is especially suitable for producing cocoa antioxidants ofr use in pharmaceutical, cosmetic and nutritional compositions.

EP 1 026 164 A1

Description

[0001] The present invention relates to a process for producing polyphenolic antioxidants with low purine content, from cocoa and other purine-containing plant materials.

[0002] Polyphenolic antioxidants from plant materials derived from cocoa, coffee, tea, and other theobroma species are interesting as they are assumed to be active in preventing cancer, coronary disease and strokes and in delaying aging processes. These antioxidants are usually water-soluble and comprise compounds of the chroman type, such as catechin and epicatechin (the stereoisomeric 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromans), and dimerised structures including procyanidin. However, these plant materials also contain purines such a caffeine (1,3,7-trimethyl-2,6-purinedione, 1,3,7-trimethylxanthine) and theobromine (3,7-dimethyl-2,6-purinedione, 3,7-dimethylxanthine), and these components are usually undesired in antioxidant compositions because of their stimulating properties.

[0003] Current processes for extracting polyphenols from cocoa comprise treatment of defatted cocoa material with acetone, water/methanol, chloroform and ethyl acetate. Such a process is described in WO 98/09533 (Mars Inc.). A process for removing purines (theobromine and caffeine) from cocoa material by extraction with water of 45-55°C followed by extraction with water of about 100°C is described in US 4,755,391 (Hershey Foods Corp.).

[0004] Methylxanthines (purines) are extracted with chlorinated hydrocarbon solvents, such as chloroform, ethylene dichloride or tetrachloroethane. Methylxanthines are also removed by adsorption on an adsorption material like activated carbon, carob particles or a cation exchange resin as described in US 4,956,429, US 4,390,698 and US 4,444,798.

[0005] The problem to be solved with the present invention was to provide a process for producing a natural, fat-free, water-soluble polyphenolic antioxidant composition having a maximum antioxidant activity and a minimum purine content, said process being technically and economically feasible without the use of objectionable chemicals.

[0006] The process of the invention is characterised by the features of the appending claims, and comprises the steps of:

(a) extracting the plant material with an hydroxylic extracting liquid, preferably at a temperature below 50°C;
(b) adsorbing the extract obtained in step (a), preferably at a temperature below 30°C;
(c) desorbing the adsorbed material with a hydroxylic desorbing liquid, preferably at a temperature above 30°C, and
(d) concentrating and optionally drying the desorbed material.

[0007] The process of the invention can be used for extracting polyphenolic antioxidants from purine-containing materials, in particular, tea and tea derivatives, coffee beans and derivatives thereof and cocoa beans and cocoa derivatives. The latter include beans from other theobroma species like *Theobroma grandiflora*. Coffee and especially cocoa products are the preferred starting materials. The starting cocoa products may be e.g. fresh beans, defatted solids, comminuted trash beans, cocoa powder, low-fat cocoa powder, cocoa shells, cocoa waste or other raw materials. The starting material is preferably ground to a particle size below 250 µm.

[0008] An essential feature of the present invention is the use of an adsorption material that has a high polyphenol/purine adsorption selectivity. This relative selectivity, expressed as polyphenolic antioxidant / purine adsorption ratio for a hydroxylic plant extract, is in particular at least 5/1, especially at least 9/1. Under selected conditions, the adsorbent thus has a minimum adsorption of the methyl-xanthines and a maximum adsorption of the polyphenolic antioxidants from the extract. The polyphenolic antioxidants thus selectively adsorbed are subsequently desorbed and concentrated. The present invention results in the production of a high grade antioxidant composition having a low purine content without the use of questionable chemicals and solvents; residues of these are most undesirable in medicinal, nutraceutical, nutritional and pharmaceutical applications.

[0009] The adsorbent to be used for selectively adsorbing polyphenolic compounds is preferably polyvinylpolypyrrolidone (PVPP), i.e. crosslinked polyvinylpyrrolidone, or a derivative or modification thereof. Examples of chemical modification of PVPP are the common nucleophilic substitution reactions on the carbonyl group like reactions with alcohols (acetal formation), amines (Schiff base formation) and phosphorous ylids (Wittig reaction), and the reduction of the carbonyl group using $LiAlH_4$. The purpose of the modification is to modify the adsorption characteristics of PVPP by changing the hydrophobicity of the PVPP material. Examples of physical modification of PVPP are controlled expansion using liquified or supercritical gases, grinding or solvent treatment of the PVPP matrix. The purpose of such treatments is to increase the surface area per gram of material and/or to modify gravimetric separation properties when using the PVPP in a combined extraction/adsorption step. PVPP of the commercially available grade is suitable. Other suitable adsorbents include chitosan and blends between PVPP and chitosan or chemical of physical derivatives thereof. The amount of adsorbent to be used can be e.g. between 0.2 and 200 g, preferably between 5 and 50 g per liter of extract.

[0010] Prior to the adsorption step, the polyphenolic antioxidants are extracted from the plant material using a hydroxylic solvent, preferably water. The temperature of the extraction can be varied. A significant characteristic of the

process of the invention is that if the plant material is extracted at low temperatures, a minimal extraction of purines and maximal extraction of the antioxidants is obtained. Thus the selectivity of the process of the invention can be further increased by using low extraction temperatures, i.e. below 50°C, especially below 30°C.

[0011] The hydroxylic solvent to be used according to the invention is a solvent which selectively extracts polyphenolic compounds. It is selected from water and lower alcohols, such as methanol, ethanol, propanol, isopropanol, one of the isomeric butanols, methoxyethanol, glycol and the like. The preferred extracting liquid is water or ethanol or a mixture thereof, most preferred is water. The preferred desorbing liquid is water or ethanol a or mixture thereof. The amount of extracting liquid can be e.g. between 5 and 50 times (w/w) the amount of starting plant material. The desorption can be carried out at a pH between 4 and 9, especially at about neutral pH (5.5-8).

[0012] The desorbed material can subsequently be concentrated. The main part of the desorption liquid can be evaporated (under partial vacuum if necessary). The antioxidant active material can then be dried using freeze-drying and/or vacuum distillation.

[0013] The antioxidant activity of the product can be expressed in the capability to scavenge free radicals. This capacity can be expressed towards Trolox, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, a water-soluble variant of vitamin E, in TEAC (Trolox Equivalent Antioxidant Capacity) as is also presented by Salah et al, *Archives of Biochemistry and Biophysics*, **322**, (1995), no 2, pp. 339-346.

[0014] The antioxidant composition that can be obtained with the process of the invention has at least a TEAC value of 0.4 and less than 5% by weight of purine components. In particular, the composition obtainable by the process of the invention has at least a TEAC of 0.4 and less than 3.5% by weight of purine components. In particular it contains at least 15%, especially at least 25% by weight of polyphenolic antioxidants. It is free of (i.e. contains less than 10 ppm of) chlorinated hydrocarbons and is substantially fat-free (i.e. contains less than 0.5 wt.% of fat).

[0015] The antioxidant product can be used as an ingredient for nutritional products and can be used as a basic substance for e.g. chocolate bars, beverages, confectionary, ice-cream, pastries. The product can also be used in medicinal, nutraceutical, pharmaceutical and cosmetic (e.g. skin care) formulations.

[0016] The process of the invention is further illustrated by the flow diagram in the accompanying figure. The process for the production of antioxidants consists of a (consecutive or counter-current) extraction of plant material with a hydroxylic solvent followed by separation of the extract. Separation may be achieved by filtration, ultrafiltration or centrifugation, depending on the desired clarification and purity. After separation the extract should be clear and free of solid or colloidal particles. The extract is then subjected to an adsorption section where the polyphenolic antioxidants are adsorbed on adsorption material. The hydroxylic solvent with the unadsorbed purines can be recycled. Another possibility is the combination of extraction and adsorption with the addition of the material to the extract/solids mixture and subsequent physical separation of the adsorption material from this mixture. Desorption also takes places with a hydroxylic solvent. Desorption can be done by placing the adsorption material in a column and then performing (consecutive) washings or bed spraying. Another desorption technique is immersing extraction of the adsorption material. The desorbed solution of active components may be further concentrated and/or dried as desired by any technical means available, like evaporation whether or not under reduced pressure, vacuum distillation, reversed osmosis, freeze-drying or spray-drying.

[0017] **Example 1**: *Immersion extraction of cocoa-powder at different temperatures.* In a 1000 ml conical flask 50 grams of cocoa powder, of the N-11-N type, containing 10.5% fat was mixed with 500 gram of demineralised water. The set point temperature was maintained with a temperature controller. Homogeneous samples of 10 ml were taken after 15 minutes. The extraction was conducted at 20, 40, 60, 80, and 100 °C. The concentrations of theobromine (THB), caffeine (CAF), catechin (CAC), and epicatechin (EPC) in the samples were analysed with a HPLC, using a Waters symmetric 4.6x250 mm C18 15µm column, a photodiode array detector at 276 nm, a flow rate of 1.5 ml/min and a column temperature of 35°C. The mobile phase was a varying gradient 2.5 vol.% acetic acid and 30 vol.% acetonitrile in water.

Table 1:

Influence of temperature on the extraction of cocoa (in g/l)				
Temperature (°C)	THB	CAF	CAC	EPC
20	1.21	0.06	0.15	0.10
40	1.73	0.06	0.14	0.09
60	2.17	0.07	0.15	0.10
80	2.39	0.07	0.14	0.08
100	2.43	0.07	0.15	0.08

[0018] Table 1 shows the surprising tendency that the extraction performance of the antioxidants does not significantly

vary within the temperature range of 20-100°C. The undesired purines, mainly theobromine, display the lowest extraction performance at 20°C. Therefore, an extraction temperature below 30 °C will result in a minimum extraction of purines and a maximum extraction of antioxidants, for the described type of cocoa powder.

Example 2: *Adsorption of a cocoa extract on PVPP followed by desorption and freeze-drying*

[0019] Natural cocoa powder, which is rich in catechines, was extracted with demineralised water at 20°C for three hours at a ratio of 10 w/w. An amount of 400 gram extract was mixed (magnetic stirrer) with 4 gram crosslinked PVPP (CAS 25249-54-1 obtained from Akcros) at 5°C during 90 min. The adsorbed material was then separated using a glass filter (por. 0). This was placed in a Soxhlet apparatus and refluxed with demineralised water for 24 hours. Subsequently, the extract was subjected to freeze-drying, resulting in a dry cocoa material enriched in antioxidant activity. The concentrations of theobromine (THB), caffeine (CAF), catechin (CAC), and epicatechin (EPC) in the samples were analysed by HPLC. The antioxidant activity was measured using the DPPH radical scavenging method (Brand-Williams et al., *Lebensmittel-Wissenschaft und Technologie*, **28**, 1995, pp. 25-30). This method measures the adsorbance before and after (4 hours) the addition of an antioxidant to an ethanolic solution of DPPH radicals. This radical has a deep purple colour and absorbs at 515 nm. Trolox was used as reference antioxidant and the TEAC values are based on the amount (in g) of antioxidant-active material that equals the amount (in g) of Trolox necessary for scavenging 50% of the DPPH radicals. Trolox and DPPH were obtained from Fluka Chemika.

Table 2:

Performance of the adsorption				
	THB	CAF	CAC	EPC
Adsorption efficiency [%]	1	10	100	96

[0020] Table 2 shows the adsorption efficiency of the adsorption of the four relevant cocoa components theobromine, caffeine, catechin and epicatechin from the cocoa extract with PVPP. Bringing the aqueous cocoa extract in contact with the PVPP adsorbent results in a surprisingly low interaction of the purines with the adsorbent and in a selective adsorption of catechin and epicatechin. Table 3 shows the composition of the initial cocoa material, and the final product composition in the final product after freeze-drying, including TEAC values. The "other" material also comprises polyphenolic antioxidants (presumably about 20 wt.%).

Table 3:

Overall performance of antioxidant production						
	THB [wt.%]	CAF [wt.%]	CAC [wt.%]	EPC [wt.%]	other [wt.%]	TEAC [g]
Starting composition [wt.%]	3	0.3	0.3	1.2	95	0.05
Product composition [wt.%]	3	0.4	19	11	67	1.0

Claims

1. A process for isolating polyphenolic antioxidants from purine-containing plant material, comprising the steps of

   (a) extracting the plant material with a hydroxylic extracting liquid;
   (b) adsorbing the extract obtained in step (a) on a solid adsorbent which has a high polyphenol/purine adsorption selectivity;
   (c) desorbing the adsorbed material with a hydroxylic desorbing liquid, and
   (d) concentrating and optionally drying the desorbed material.

2. A process according to claim 1, in which the adsorbent comprises polyvinyl-polypyrrolidone or a chemical or physical modification thereof.

3. A process according to claim 1 or 2, in which the adsorbent comprises chitosan or a chemical or physical modification thereof.

4. A process according to any one of the preceding claims, in which step (a) is carried out at a temperature below

50°C, preferably between 0 and 30°C.

5. A process according to any one of the preceding claims, in which step (b) is carried out at a temperature below 30°C, preferably between 0 and 20°C.

6. A process according to any one of the preceding claims, in which step (c) is carried out at a temperature above 30°C, preferably between 50 and 110°C.

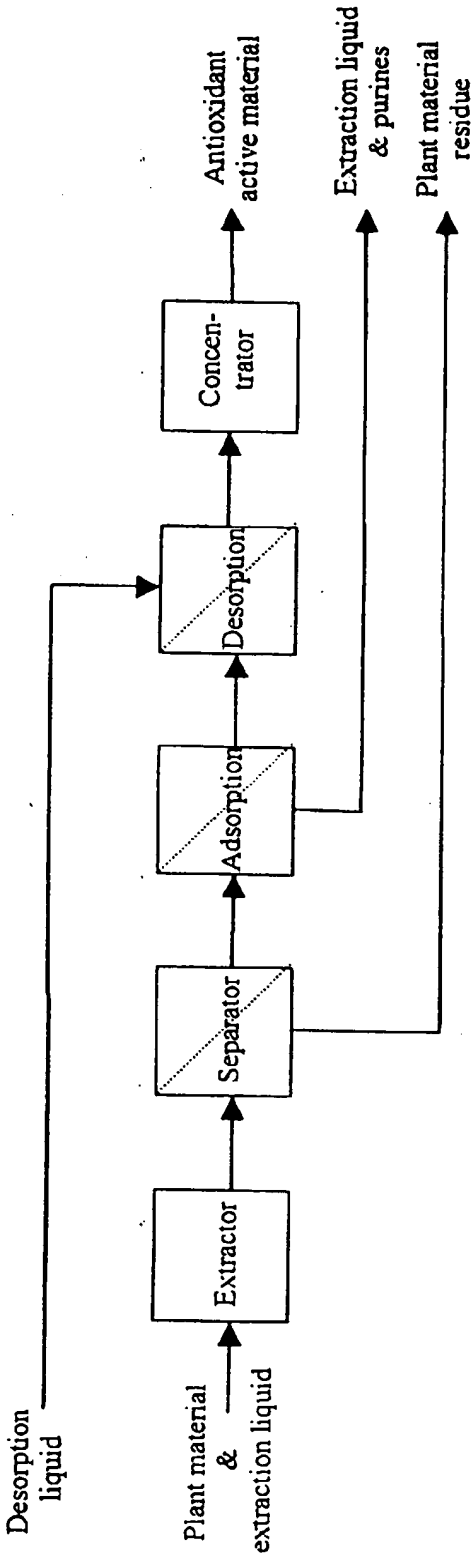
7. A process according to any one of the preceding claims, in which the hydroxylic extracting liquid in step (a) comprises water.

8. A process according to any one of the preceding claims, in which the hydroxylic desorbing liquid in step (c) comprises water and/or a lower alcohol.

9. A process according to any one of the preceding claims, in which said purine-containing plant material is cocoa, coffee or tea, especially cocoa.

10. A water-soluble antioxidant composition obtainable using the process of any one of claims 1-9, which is free of chlorinated hydrocarbons and substantially fat-free, and which contains at least 15% by weight of polyphenolic antioxidants and less than 5% by weight of purine components, and has a TEAC value of at least 0.4.

11. A nutritional, pharmaceutical or cosmetic composition containing an antioxidant composition according to claim 10.


Desorption liquid
Plant material & extraction liquid
Extractor
Separator
Adsorption
Desorption
Concen-trator
Antioxidant active material
Extraction liquid & purines
Plant material residue

Figure

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EUROPEAN SEARCH REPORT

Application Number
EP 99 20 0301

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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X	PATENT ABSTRACTS OF JAPAN vol. 098, no. 008, 30 June 1998 & JP 10 067771 A (NIPPON YOURIYOKUSO KK), 10 March 1998 * abstract * & DATABASE WPI Derwent Publications Ltd., London, GB; AN 98-225186 * abstract *	1-11	
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
MUNICH	19 July 1999	Villa Riva, A

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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EUROPEAN SEARCH REPORT

Application Number
EP 99 20 0301

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
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Y	PATENT ABSTRACTS OF JAPAN vol. 097, no. 012, 25 December 1997 & JP 09 220053 A (ITOUEN:KK), 26 August 1997 * abstract *	1-9	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)

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